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FORMES MULTIPLES DE LYSOZYMES : STRUCTURE PRIMAIRE ET SITE ACTIF DE DEUX LYSOZYMES DU BLANC D'OEUF DE CANE

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Multiple Forms of Duck-Egg-White Lysozyme

Primary Structure of Two Duck Lysozymes

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Lysozyme was purified from pooled samples of Peking, Kaki and "wild-type" duck egg-whites by gel filtration and ion-exchange chromatography on Amberlite CG-50. Two different lysozymes were found, called in relation with previous studies, duck-lysozyme II and duck-lysozyme III. The enzymes were shown to be pure by several criteria. The complete primary structures of the two duck lysozymes were established. The reduced and alkylated enzymes were subjected to digestion with trypsin. The structures of all the tryptic peptides were established. For both lysozymes, alignment of the tryptic peptides into a single chain containing 129 amino acids was determined from basic overlap peptides obtained by digestion of the enzyme with chymotrypsin. Six replacements were noted between the two duck lysozymes. Further comparisons were achieved with hen egg-white and other lysozymes; the possibility of the existence of multiple alleles at one lysozyme locus is suggested for explaining the presence of multiple duck lysozymes.

Only a single type of lysozyme was found in hen, guinea-hen and goose egg-white as well as in normal human tissues and secretions [1]. Chemical-structure studies by Jollès *et al.* concerning hen [2] and guinea-hen [3] egg-white and human milk [4,5] lysozymes demonstrated extensive sequence similarities. However the goose lysozyme [6,7] is not only quite different from these three enzymes but also from duck lysozymes; this is rather surprising since the duck and goose are relatives in the same bird family (Anatidae).

Multiple lysozymes have been found in duck egg-white and similar observations have been made with lysozymes purified from tissues or secretions of patients suffering from leukemia [8] (leucocytes from patients with chronic myelogenous leukemia). From pooled duck egg-whites, Jollès *et al.* in 1965 [9], characterised by ion-exchange chromatography on Amberlite CG-50 three peaks, called I, II and III, containing material active against *Micrococcus lysodeikticus* cells. From peaks II and III, histidine-free duck lysozymes, were isolated and partially studied [9,10]; their amino-acid composition [9,10], their electrophoretical [9] and immunological [11] behaviours and their behaviour in the presence of inhibitors [12] were slightly different. Imanishi *et al.* [13] in 1966 and Prager and Wilson [14] in 1971, reported also the presence of multiple lysozymes in the duck egg-white, two (DL-1, DL-2) and three (A, B, C from pooled egg-white of the Peking duck), respectively. A preliminary report indicating the complete primary-structure of duck egg-white lysozyme II has been published by Hermann and Jollès [15].

The present paper deals with the purification of egg-white lysozymes from three particular species of duck and with the detailed study concerning the establishment of the primary structure of duck egg-white lysozymes II and III.

MATERIAL AND METHODS

Peking, Kaki and "wild-type" duck eggs, freshly laid, were obtained from the *Centre de Sélection et d'Elevage des Animaux de Laboratoire du C.N.R.S.* The egg-whites were separated from the yolks and stored frozen.

Hen egg-white lysozyme, trypsin, chymotrypsin and carboxypeptidases A and B were obtained from Worthington. Aminopeptidase O was a gift from Dr. H. Uhlig (Röhm u. Haas, Darmstadt). Sephadex G-25 fine and G-75 fine were obtained from Pharmacia and Amberlite CG-50 (type II) and Dowex 1X2 (200—400 mesh) from Touzart and Matignon (Paris). All other reagents were purchased from Merck or Prolabo.

Lysis of *Micrococcus luteus* (formerly named *M. lysodeikticus*) was measured according to the procedure of Jollès [16]; the absorbance at 280 nm was used to measure protein.

This is 79th communication on lysozymes, for 78th communication see [3].

Enzymes. Lysozyme (EC 3.2.1.17); trypsin (EC 3.4.4.4); chymotrypsin (EC 3.4.4.5); carboxypeptidases A and B (EC 3.4.2.1 and 3.4.2.2).

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Purification of the Enzyme

The purification procedure of duck-egg-white lysozyme was based upon published methods [9, 17]. Duck egg-white from 12 eggs of a well-defined species was diluted 1:5 in water and the pH was adjusted at pH 4.5 with 30% acetic acid. The precipitate formed was removed by filtration. 60 ml of Amberlite CG-50 equilibrated in a 0.2 M phosphate buffer pH 6.5 were added to the clear supernatant and the suspension was stirred at 20 °C during 6 h. The rest of the purification (except the dialysis) was carried out at room temperature. The resin with the lysozyme bound to it was washed with water and with 0.2 M phosphate buffer pH 6.5. The lysozyme was eluted with a 0.8 M phosphate buffer pH 6.5. After a short dialysis (1—2 h) against distilled water (4 °C), the biologically active material was subjected to two gel filtrations on Sephadex G-25 (100×3 cm, 0.1 N acetic acid) and Sephadex G-75 (100×2 cm, 0.1 N acetic acid) successively. The biologically active material was further purified by ion-exchange chromatography on Amberlite CG-50 (a quite important step in our purification procedure), desalted on Sephadex G-25 and evaporated in a dessiccator.

Analytical Methods

Acrylamide gel electrophoresis was performed at pH 2.9, 90 volts, 5 mA for 30 min.

The amino-acid composition of the peptides was established with a Technicon AutoAnalyzer after total hydrolysis (6 N HCl, 110 °C, 18 h, under vacuum).

The different methods employed throughout this research for the determination of the primary structure of the two duck lysozymes have recently been discussed in detail by Jollès and Jollès [18]. Summarizing, each lysozyme (240 mg) was reduced with 2-mercaptoethanol and carboxymethylated. It was then digested with 1% (w/w) of trypsin at 37 °C, pH 7.8 for 24 h. Trypsin was pretreated during 16 h with 0.0625 N HCl at 37 °C. The peptides obtained after tryptic hydrolysis were separated by column chromatography on Dowex 1X2. The peptides obtained after chymotryptic hydrolysis (2%, w/w of enzyme, pH 7.8, 37 °C, 24 h) were separated on Dowex 1X2 or Sephadex G-25. When several peptides were characterized in a peak, their purification was achieved by preparative paper-chromatography (Whatman No. 1, *n*-butanol—formic acid—water, 75:15:10, v/v/v) or paper electrophoresis at pH 6.5 (Whatman No. 1, pyridine—water—acetic acid, 100:900:4, v/v/v, 50 V/cm). The indicated yields are calculated after chromatography on Dowex 1X2. The structures of the peptides were established by the Edman-dansyl technique, carboxypeptidase and aminopeptidase digestions, chymotryptic digestion of a tryptic peptide, tryptic digestion of a chymotryptic peptide when possible, partial acid hydrolysis, *etc.*

Table 1. *Preparation of lysozymes II and III from the Kaki duck egg-white*

Purification step	Lysozyme (expressed as hen lysozyme)
	mg
1. *Preparation of a purified material*	
Dilution of egg-white from 12 eggs in 5 vol. water	330
Adsorption onto Amberlite CG-50 in 0.2 M phosphate buffer pH 6.5 (5 ml resin/egg) Elution with 0.8 M phosphate buffer, pH 6.5, after washing with water	308
Desalting by dialysis and Sephadex G-25 filtration (100×3 cm, 0.1 N acetic acid)	300
2. *Elimination of high molecular-weight inactive substances*	
Filtration on Sephadex G-75 (100×2 cm, 0.1 N acetic acid) "Purified" lysozyme put onto the column	69
Lysozyme obtained in the active peak	48
3. *Preparation of chromatographically-pure lysozyme samples*	
Ion-exchange chromatography on Amberlite CG-50 (pH 7.56, 0.2 M phosphate buffer, 80×0.9 cm)	
"Purified" lysozyme put onto the column	32
Chromatographically pure fractions	II: 13 III: 14
Desalting of each peak on Sephadex G-25 (100×3 cm, 0.1 N acetic acid)	10 11

RESULTS

Purification of Lysozymes from the Egg-White of Different Duck Species

The quantitative preparation of lysozyme from 12 Kaki duck eggs is summarized in Table 1. Only during the last purification step, the ion-exchange chromatography on Amberlite CG-50, was it possible to characterize two biologically active peaks (Fig. 1), corresponding to enzymes II and III formerly described [9]. Peak I, previously eluted with the dead volume when pooled egg-white from different species was employed, was not present. Each of the two duck lysozymes tested emerged at the same place as a single symmetrical peak when rechromatographed on the same column of Amberlite CG-50. The specific activity was constant across the peak. In addition, each lysozyme moved as a single protein zone upon starch-gel electrophoresis and arcylamide-gel electrophoresis and had a slightly different electrophoretic mobility.

Similar results were obtained when lysozyme was isolated from Peking and wild-type duck eggs.

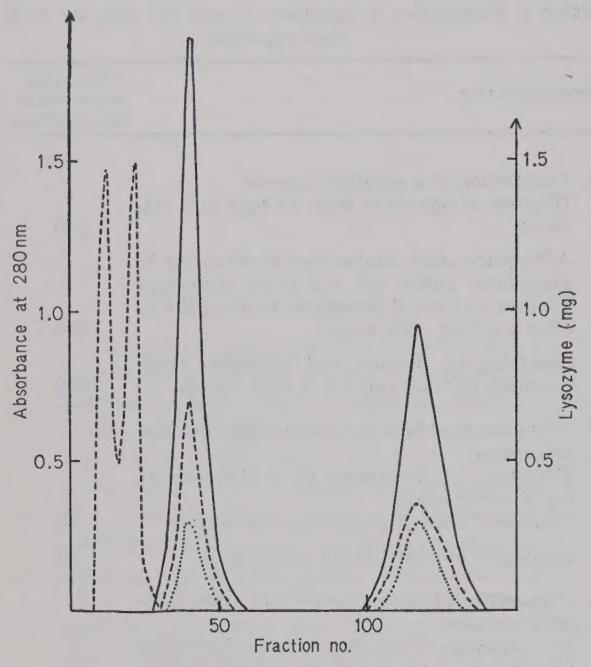

Fig. 1. *Ion-exchange chromatography on Amberlite CG-50 (80×0.9 cm), pH 7.56, 0.2 M sodium-phosphate buffer, of 40 mg "purified" Kaki-duck-egg-white lysozyme (quantity expressed as hen-egg-white lysozyme).* 3-ml fractions were taken. ----, absorbance at 280 nm; ——, enzymic activity (mg lysozyme expressed as hen lysozyme); ·····, enzymic activity when each active peak (about 10 mg) was rechromatographed separately on the same column

Peaks II and III emerged at the same place as the active fractions from Kaki egg-whites when chromatographed on Amberlite CG-50. The ratio between enzymes II and III established after complete purification, was comprised between 1:2 and 2:1 and did not seem to be constant.

Under our experimental conditions, the duck lysozymes did not differ significantly from one another or from hen lysozyme in their capacity to lyse *M. luteus*.

Primary Structure of Duck-Egg-White Lysozyme II

N- and C-Terminal-Amino-Acid Sequences. By the Edman-dansyl technique, Lys-Val-Tyr was characterized as the N-terminal sequence of the enzyme. Leucine was the unique amino acid liberated by the action of carboxypeptidase A.

Tryptic Peptides. After chromatography of the tryptic hydrolysate on Dowex 1X2, 13 peaks (T1—T13) were characterized by the ninhydrin reagent after alkaline hydrolysis. In Table 2 the R_F and mobility (m) values are indicated, as well as the yields and the structures of the tryptic peptides accounting for all the amino acids present in the molecule.

Chymotryptic Peptides and Alignment of the Tryptic Peptides. The chymotryptic digest was submitted to a chromatography on Dowex 1X2 (140×1.5 cm) and 24 fractions were characterized. The different chymotryptic peptides contained in these fractions were purified and their structures, accounting for all the amino acids present in the molecule, are indicated in Table 3.

The chymotryptic peptides containing basic amino-acids were used in reconstructing the complete amino-acid sequence of duck egg-white lysozyme II (Table 6).

Primary Structure of Duck-Egg-White Lysozyme III

Terminal Amino Acids. Duck II and duck III lysozymes have the same terminal amino acids.

Tryptic Peptides. The tryptic peptides of duck-lysozyme III were purified and studied as those of duck-lysozyme II. Table 4 reports the structure of the six tryptic peptides which are different from the corresponding peptides of duck-lysozyme II.

Chymotryptic Peptides and Alignment of the Tryptic Peptides. The chymotryptic peptides of duck-lysozyme III were purified by filtration on Sephadex G-25 (200×2 cm) with 30% acetic acid as eluent. 19 fractions were characterized. The peptides were purified by the usual procedures and their structures accounting for all the amino acids present in the molecule are indicated in Table 5. Peptide C8 was found under two different forms (see Discussion): Glu-Ser-Ser-Phe (70%) and Glu-Ser-Gly-Phe (30%).

The chymotryptic peptides containing basic amino-acids were employed in establishing the complete amino-acid sequence of duck egg-white lysozyme III (Table 6).

DISCUSSION

The presence of multiple lysozymes in duck egg-white of different species was established by various experimental procedures and by different authors as mentioned in the Introduction. But for the first time the complete primary structure of two biologically active fractions, II and III, each of which emerged at the same place as a single symmetrical peak when rechromatographed on Amberlite CG-50, was determined. The chromatographic behaviour of these duck-lysozyme fractions II and III was the same when obtained from Peking, Kaki or wild-type duck egg-whites. Six amino-acid differences exist between duck-lysozymes II and III. The replacement of a residue of glycine by a residue of arginine (residue No. 71) has to be mentioned particularly, as the introduction of a new basic amino-acid might explain the immunological difference between the two enzymes [11]. The number of amino-acid replacements is of the same order as that observed between lysozymes from different bird egg-whites [3]. As an

Table 2. R_F-*values, mobility* m *(at pH 6.5), yields and chemical structure of the tryptic peptides of reduced and alkylated duck egg-white lysozyme II*

The amino acids printed in bold-face type are different from those occurring in the corresponding peptides of hen lysozyme; differences in the amidation of aspartate, asparagine and glutamate residues are indicated similarly, in peptide T11

Peptide	R_F	m [a]	Yield	Structure
			%	
T 1b	0.05	+1.10	40	Lys
T 3a	0.12	+0.50	36	Val-**Tyr**-**Ser**-Arg
T 5 [b]	0.02	0	34	SCMCys-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg
T 8c [b]	0.16	−0.32	14	SCMCys-Glu-Leu-Ala-Ala-Ala-Met-Lys
T 8b	0.27	0	54	**Leu**-Gly-Leu-Asp-Asn-Tyr-Arg
T13a	0	−0.14	10	Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-SCMCys-Ala-Ala-**Asn**-**Tyr**-Glu-Ser-**Ser**-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg
T11	0	−0.46	25	**Asn**-Thr-**Asp**-Gly-Ser-Thr-Asp-**Tyr**-Gly-Ile-Leu-**Glu**-Ile-Asn-Ser-Arg
T10 [c]	0	0	45	Trp-Trp-SCMCys-Asp-Asn-Gly-**Lys**-Thr-Pro-Gly-Ser-**Lys**
T13b [c]	0.10	−0.27	10	Trp-Trp-SCMCys-Asp-Asn-Gly-**Lys**
T 2a [c]	0.03	+0.50	10	Thr-Pro-Gly-Ser-**Lys**
T 9	0.27	−0.35	43	Asn-**Ala**-SCMCys-**Gly**-Ile-Pro-SCMCys-Ser-**Val**-Leu-Leu-**Arg**
T 8a	0.16	−0.32	51	Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-**Arg**
T 7b	0.12	0	60	SCMCys-Ala-Lys
T 1a	0.08	+1	92	**Arg**
T12	0.02	−0.30	40	Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp-Val-Ala-Trp-Arg
T 2b	0.03	+0.63	53	Asn-Arg
T 7a	0.12	0	43	SCMCys-**Arg**
T 4b	0.03	0	53	Gly-Thr-Asp-Val-**Ser**-**Lys**
T 4a	0.28	+0.52	36	Trp-Ile-Arg
T 6 [d]	0.16	0	42	Gly-SCMCys-Arg-Leu
T 7c [d]	0.06	0	10	Gly-SCMCys-Arg
T 3b [d]	0.70	0	30	Leu

[a] $m = 0$ for Gly; $m = +1$ for Arg; $m = -1$ for $CySO_3H$.
[b] T 5 = T 8 c + Arg.
[c] T 10 = T 13b + T 2a.
[d] T 6 = T 7c + T 3b.

Table 3. R_F, *mobility* m *(at pH 6.5) and structure of the chymotryptic peptides of duck-egg-white lysozyme II*

The peptides are indicated in this table in the order as they occur in the molecule. The amino acids in bold-face type are different from those occurring in the corresponding peptides of hen lysozyme; differences in the amidation of aspartate, asparagine and glutamate residues between these two lysozymes are indicated similarly

Peptides	R_F	m	Structure
1	0.16	+0.40	Lys-Val-**Tyr**
2	0.12	−0.32	**Ser**-Arg-SCMCys-Glu-Leu
3	0	+0.65	Ala-Ala-Ala-Met-Lys-Arg-**Leu**
4	0.27	−0.40	Gly-Leu-Asp-Asn-Tyr
5	0.12	+0.45	Arg-Gly-Tyr
6	0.40	0	Ser-Leu-Gly-Asn-Trp
7	0.16	−0.30	Val-SCMCys-Ala-Ala-**Asn**-**Tyr**
8	0.22	−0.40	Glu-Ser-**Ser**-Phe
9	0	0	Asn-Thr-Gln-Ala-Thr-Asn
10	0	−0.25	Arg-**Asn**-Thr-**Asp**-Gly-Ser-Thr-Asp-Tyr
11	0.78	0	Gly-Ile-Leu
12	0.27	0	**Glu**-Ile-Asn-Ser-Arg-Trp
13	0	−0.50	Trp-SCMCys-Asp-Asn-Gly-**Lys**-Thr-Pro-Gly-Ser-**Lys**-Asn-**Ala**-SCMCys-**Gly**-Ile-Pro-SCMCys-Ser-**Val**-Leu
14	0	−0.25	Leu-**Arg**-Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-**Arg**-SCMCys-Ala-**Lys**-**Arg**-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp
15	0.60	0	Val-Ala-Trp
16	0	+0.5	Arg-Asn-Arg-SCMCys-**Arg**-Gly-Thr-Asp-Val-**Ser**-Lys-Trp
17	0.10	+0.36	Ile-Arg-Gly-SCMCys-Arg-Leu

example, the differences noted between duck II, duck III, guinea-hen, turkey and quail lysozymes on one side and hen egg-white lysozyme on the other side are 19, 18, 10, 6 and 6, respectively [2, 3, 15, 19, 20]. Although a number of reported allelic differences, such as those for human hemoglobin [21], appear to result in single amino-acid differences, cases of multiple amino-acid differences have been noted, as for the β-chains of sheep hemoglobins A and B, which differ by at least 7 residues [22]. Recent anal-

Table 4. R_F *and mobility* m *(at pH 6.5) values and structure of the tryptic peptides of duck lysozyme III absent in duck lysozyme II*
The corresponding peptide of duck lysozyme II is indicated between parentheses (see Table 2). Amino acids printed in bold-face type are different from the corresponding amino acid of hen lysozyme, those in italic are different from the corresponding amino acid of duck lysozyme II

Peptide	R_F	m	Structure
1 (T1b + T3a)	0.02	+0.40	Lys-Val-**Tyr**-*Glu*-Arg
2 (part of T2a)	0.08	+0.56	Thr-Pro-*Arg*
3 (part of T2a)	0.08	+0.65	*Ala*-Lys
4 (T8a)	0.10	−0.28	Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-*Lys*
5 (T7a)	0.08	0	SCMCys-*Lys*
6 (T4b)	0.03	0	Gly-Thr-Asp-Val-**Ser**-*Arg*

Table 5. R_F *and mobility* m *(at pH 6.5) values and structure of the chymotryptic peptides of duck lysozyme III*
The peptides are indicated in this table in the order as they occur in the molecule. Amino acids printed in bold-face type are different from the corresponding amino acid of hen lysozyme, those in italic print are different from the corresponding amino acid of duck lysozyme II

Peptide	R_F	m	Structure
1	0.16	+0.40	Lys-Val-**Tyr**
2	0.12	−0.64	*Glu*-Arg-SCMCys-Glu-Leu
3	0	+0.65	Ala-Ala-Ala-Met-Lys-Arg-**Leu**
4	0.27	−0.40	Gly-Leu-Asp-Asn-Tyr
5	0.12	+0.45	Arg-Gly-Tyr
6	0.40	0	Ser-Leu-Gly-Asn-Trp
7	0.16	−0.30	Val-SCMCys-Ala-Ala-**Asn**-**Tyr**
8	0.22	−0.40	Glu-Ser-**Ser**-Phe (70%) Glu-Ser-*Gly*-Phe (30%)
9	0	0	Asn-Thr-Gln-Ala-Thr-Asn
10	0	−0.25	Arg-**Asn**-Thr-**Asp**-Gly-Ser-Thr-Asp-Tyr
11	0.78	0	Gly-Ile-Leu
12	0.27	0	**Glu**-Ile-Asn-Ser-Arg-Trp
13	0	+0.28	Trp-SCMCys-Asp-Asn-Gly-**Lys**-Thr-Pro-*Arg*-*Ala*-Lys-Asn
14	0.27	−0.58	**Ala**-SCMCys-**Gly**-Ile-Pro-SCMCys-Ser-**Val**-Leu
15	0	−0.25	Leu-**Arg**-Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-*Lys*-SCMCys-Ala-Lys-**Arg**-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp
16	0.60	0	Val-Ala-Trp
17	0	+0.50	Arg-Asn-Arg-SCMCys-*Lys*-Gly-Thr-Asp-Val-**Ser**-*Arg*-Trp
18	0.10	+0.36	Ile-Arg-Gly-SCMCys-Arg-Leu

ysis by starch-gel electrophoresis of the partially purified lysozymes present in 44 individual duck eggs strongly suggests that the multiple forms of duck lysozyme are due to multiple alleles at a single locus [14]. In the case of the black swan (*Cygnus atratus*) however, two nonallelic genes for egg-white lysozyme appear to exist, since this species produces two lysozymes which are antigenically entirely unrelated, one rather like the hen enzyme and the other rather like the goose enzyme [23].

The existence of residue 37 in duck egg-white lysozyme III (but not in duck egg-white lysozyme II) under two forms, serine (70%) and glycine (30%) has to be mentioned; the isolation of the two corresponding chymotryptic peptides allowed this identification (Table 5). It is worth mentioning that during the structural studies devoted to hen lysozyme, Jollès (unpublished results, 1963) observed that residue 118, threonine, was in part ($\leqslant 5\%$) present as serine.

Table 6. *Primary structure of duck egg-white lysozymes II and III*
Comparison with hen egg-white lysozyme. Amino acids in bold-face type are different from the corresponding amino acid of hen lysozyme, those in italic print different from the corresponding amino acid of duck lysozyme II

Duck II	Lys-Val-**Tyr**-**Ser**-Arg-Cys-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-**Leu**-Gly-
Duck III	1Lys-Val-**Tyr**-*Glu*-Arg-Cys-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-**Leu**-Gly-
Hen	Lys-Val-Phe-Gly-Arg-Cys-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-His-Gly-
Ducl II	Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-Cys-Ala-Ala-
Duck III	17Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-Cys-Ala-Ala-
Hen	Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-Cys-Ala-Ala-
Duck II	**Asn**-**Tyr**-Glu-Ser-**Ser**-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-**Asn**-Thr-**Asp**-
Duck III	33**Asn**-**Tyr**-Glu-Ser[a]-**Ser**-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-**Asn**-Thr-**Asp**-
Hen	Lys-Phe-Glu-Ser-Asn-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-Asp-Thr-Asn-
Duck II	Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-**Glu**-Ile-Asn-Ser-Arg-Trp-Trp-Cys-
Duck III	49Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-**Glu**-Ile-Asn-Ser-Arg-Trp-Trp-Cys-
Hen	Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Trp-Trp-Cys-
Duck II	Asp-Asn-Gly-**Lys**-Thr-Pro-Gly-Ser-**Lys**-Asn-**Ala**-Cys-**Gly**-Ile-Pro-Cys-
Duck III	65Asp-Asn-Gly-**Lys**-Thr-Pro-*Arg*-*Ala*-**Lys**-Asn-**Ala**-Cys-**Gly**-Ile-Pro-Cys-
Hen	Asp-Asn-Gly-Arg-Thr-Pro-Gly-Ser-Arg-Asn-Leu-Cys-Asn-Ile-Pro-Cys-
Duck II	Ser-**Val**-Leu-Leu-**Arg**-Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-**Arg**-Cys-Ala-Lys-
Duck III	81Ser-**Val**-Leu-Leu-**Arg**-Ser-Asp-Ile-Thr-**Glu**-**Ala**-Val-*Lys*-Cys-Ala-Lys-
Hen	Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-Lys-
Duck II	**Arg**-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp-Val-Ala-Trp-Arg-
Duck III	97**Arg**-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp-Val-Ala-Trp-Arg-
Hen	Lys-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp-Val-Ala-Trp-Arg-
Duck II	Asn-Arg-Cys-**Arg**-Gly-Thr-Asp-Val-**Ser**-**Lys**-Trp-Ile-Arg-Gly-Cys-Arg-Leu
Duck III	113Asn-Arg-Cys-*Lys*-Gly-Thr-Asp-Val-**Ser**-*Arg*-Trp-Ile-Arg-Gly-Cys-Arg-Leu
Hen	Asn-Arg-Cys-Lys-Gly-Thr-Asp-Val-Gln-Ala-Trp-Ile-Arg-Gly-Cys-Arg-Leu

[a] Ser (70%), Gly (30%).

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